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A reagent for the non-destructive location of steroids and some other lipophilic materials on silica gel thin-layer chromatograms

There is a frequent need to locate UV non-absorbing steroids on silica gel thin-layer chromatograms by means which conserve the detected material for further investigation. It has been found that a spray reagent prepared by diluting 1 ml of a stock solution of primuline (0.1 g in 100 ml water) with 100 ml of a mixture of acetone and water (4:1) has wide application for this purpose. Primuline (Michrome No. 64), supplied by Edward Gurr Ltd., London SW14, is described in ref. 1. Merck Kieselgel precoated plates and calcium sulphate bound plates prepared in the laboratory with Machery Nagel Kieselgel proved satisfactory. Plates prepared with Machery Nagel Kieselgel bound with starch showed reduced sensitivity of detection. The primuline reagent was ineffective on partition chromatograms developed on Antec cellulose thin-layer plates. Developed silica gel chromatograms, free of developing solvent are lightly sprayed and viewed while still damp under a high pressure mercury vapour lamp with maximum output at 365 mμ (230–240 V, 125 W, Osram high pressure mercury vapour lamp, MBW/U, manufactured by GEC). A variety of both UV absorbing and UV non-absorbing steroids are visualized as pale yellow or pale blue fluorescent spots on a weakly fluorescent background. Listed below are some representative steroids which have been detected, with their approximate sensitivity limits on Merck Kieselgel precoated plates given in parentheses: pregnenolone, dehydroepiandrosterone, 5α-pregnane-3,20-dione, androsterone, 17α-hydroxypregnenolone, 3β,17β-dihydroxy-5α-androstane (0.5–1 μg); progesterone, 17α-hydroxyprogesterone, androstenedione, testosterone, 11-deoxycorticosterone, corticosterone, cortisone (1–2 μg); cortisol, oestrone, oestradiol, oestriol (2–5 μg). Oestrogen 3-methyl ethers and a number of steroid formates and acetates have also been detected satisfactorily. Sensitivity of detection diminishes as the plates dry out but may be restored by lightly spraying with acetone–water (4:1). Plates with incorporated UV-254 indicator serve as well as those without as this indicator shows no fluorescence at the longer wavelength utilized.

As an alternative procedure, plates may be predipped in the reagent and allowed to dry thoroughly before steroids are applied and the chromatograms developed. Although the sensitivity is lower than that given by the spray procedure the method has occasional advantages and sensitivity may be improved if necessary by spraying with the acetone–water mixture as described.

Preliminary experiments in which the primuline spray has been used successfully to locate: cholesterol, cholesterol oleate, 1-monopalmitin, diolein, triolein, oleic acid and the organochlorines 1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)ethane (DDT); 1,1-dichloro-2,2-bis-(*p*-chlorophenyl)ethylene (DDE); 1,1-dichloro-2,2-bis-(*p*-chlorophenyl)ethane (DDD); bis-(*p*-chlorophenyl)acetic acid (DDA) and DDA methyl ester suggest that this reagent may have widespread application for the non-destructive location of other lipophilic materials on silica gel thin-layer plates.

This work forms part of the programme of the Marine Laboratory of the Department of Agriculture and Fisheries for Scotland.

Marine Laboratory,
Department of Agriculture and Fisheries for Scotland,
Aberdeen (Great Britain)

R. S. WRIGHT

1 E. GURR, *Encyclopaedia of Microscopic Stains*, Leonard Hill, London, 1960, p. 339.

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